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Diagnosis of Koi Herpesvirus (KHV) Disease in Japan

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Abstract

The koi herpesvirus (KHV) disease was designated as a “Specific Disease” in a Japanese law amended on June 30, 2003. The Ministry of Agriculture, Forestry and Fisheries officially announced the first case of KHV disease in cultured common carp *Cyprinus carpio* in Japan in early November 2003. At that time, the Prefectural Fisheries Experimental Stations (PFESs), which belong to local government, lacked sufficient equipment and skill required to conduct a polymerase chain reaction (PCR) tests for KHV detection. Therefore, at first, most PCR tests for KHV were carried out at the National Research Institute of Aquaculture (NRIA), Fisheries Research Agency (FRA). The NRIA gave a short training course of PCR for the research staff of PFESs in mid-November. According to the established guideline for the KHV disease, the PFESs now conduct epizootic and routine clinical examinations on diseased fish followed by PCR test to detect viral DNA in the tissues. When there is a positive reaction with the PCR test, the sample is sent to the NRIA for further confirmation by PCR. Virus isolation on the KF-1 cell line is also tried, but confirmatory diagnosis is based on the results of PCR tests. By the end of 2003, the NRIA has accepted 529 individuals of 87 cases to be diagnosed and KHV-infected carp have been found in 23 out of 47 prefectures.

Key words:diagnosis, koi herpesvirus, KHV, specific disease, common carp, *Cyprinus carpio*

Diagnosis System for Exotic Diseases and Koi Herpesvirus

Some diseases are designated as “Specific Disease” in Japanese law. These are principally exotic diseases that have the potential to devastate the aquaculture industry in Japan such as spring viremia of carp (SVC) and viral hemorrhagic septicemia (VHS) of salmonid fish. For such diseases, protective guidelines have been established. The guidelines provide etiological information, diagnostic procedures, descriptions of the symptoms and other important characteristics of the disease. Laboratory diagnosis of the disease should be conducted in accordance with these guidelines.

A newly isolated herpes virus, designated as koi herpesvirus (KHV), was first reported as a causative pathogen of mass mortality among common and ornamental (koi) carp *Cyprinus carpio* cultured in Israel and the USA in 1998 (Hedrick *et al.*, 2000). A similar virus was also isolated after massive mortality of carp in Germany (Neukirch and Kunz, 2001) and Israel (Perelberg *et al.*, 2003), and the virus, isolated in Israel, was identified as carp nephritis and gill necrosis virus (CNGV) based on the histopathological results (Ronen *et al.*, 2003).

Subsequently, this viral infection has been observed in western Europe since 2000*[1], Indonesia in the spring of 2002*[2] and Taiwan in the fall of 2002 (Tu *et al.*, 2004), revealing that this disease is rapidly spreading worldwide in carp-trading countries.

In Japan, there had been no such mass mortality in carp, and KHV had not been detected by a survey conducted in Niigata Prefecture (Amita *et al.*, 2002). It has been shown that KHV is highly contagious and virulent in juvenile and adult carp (Hedrick *et al.*, 2000; Perelberg *et al.*, 2003).

Therefore, KHV infection was designated as a "Specific Disease" by a Japanese law amended on June 30, 2003, and an inspection procedure was established as a part of the guidelines (Fig. 1).

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*[1]: Le Deuff, R.-M., K. Way, L. Ecclestone, P. F. Dixon, A. M. Betts, D. M. Stone, O. Gilad, and R. P. Hedrick (2001): Abstract of the 10th International Conference of the European Association of Fish Pathologists, p 57.

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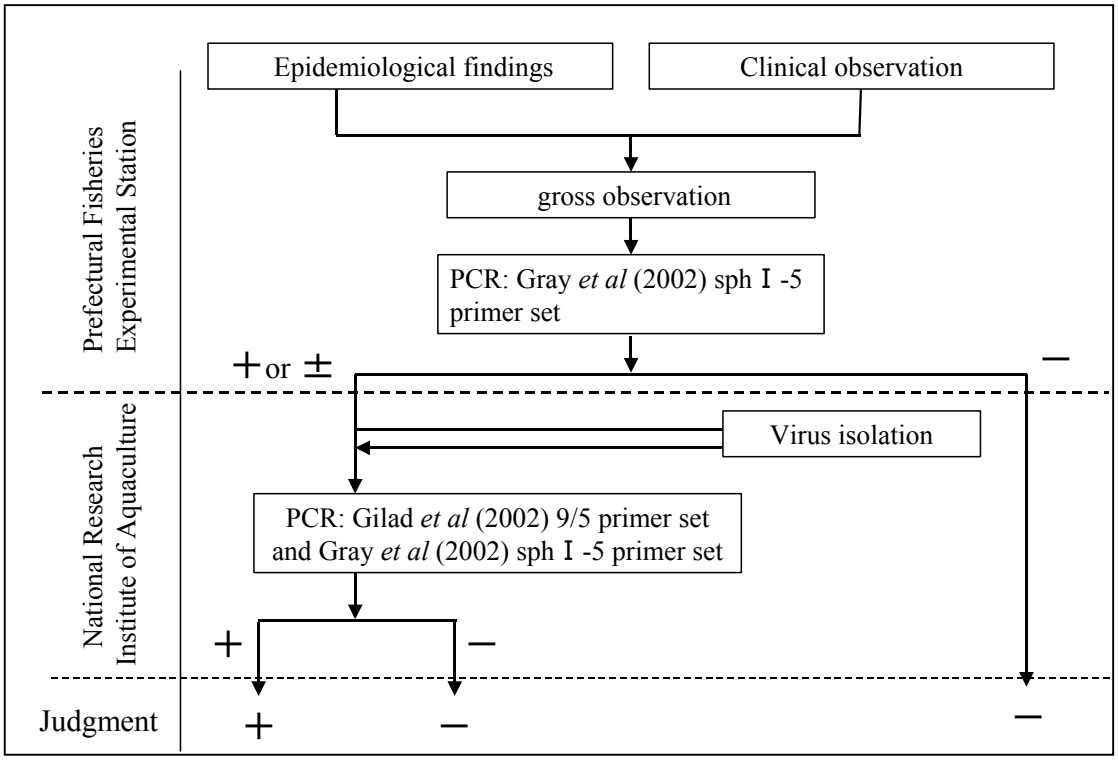

Fig. 1. Inspection procedure for KHV according to the Japanese guidelines.
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According to the procedure, the Prefectural Fisheries Experimental Stations (PFESs), which belong to local government, first conduct epizootic and routine clinical examination on diseased fish. The most important epizootiological aspect of the KHV disease is that it only affects carp *Cyprinus carpio* and occurs apparently only in a limited range of temperature from 18 to 28℃ (Hedrick *et al*., 2000; Gilad *et al*., 2003). Therefore, the water temperature and affected species of fish should be determined during a field examination.

Few external signs are usually visible, but pale and necrotic gills are frequently evident. *Flavobacterium columnare* (=*Flexibacter columnaris*) infection and some protozoan parasites, such as *Chilodonella* and *Trichodina*, are sometimes found on necrotic gill lesions, easily leading to misdiagnoses.

If any doubt remains as to the presence of KHV, a polymerase chain reaction (PCR) test can be used to detect KHV DNA in the tissues of fish. The PCR method described by Gray *et al*. (2002) was adopted in the inspection procedure as the primary examination to be conducted by the PFESs.

When the PCR test was positive for KHV, the sample is sent to the National Research Institute of Aquaculture (NRIA), Fisheries Research Agency (FRA), for further examination by the PCR methods of both Gilad *et al*. (2002) and Gray *et al*. (2002) for confirmation. Virus isolation on the KF-1 cell line is also attempted using the KF-1 cell line (Hedrick *et al*., 2000). Because of the difficulty of isolating the virus in the cell line, the results of the isolation trial is treated as supplementary data, and confirmation of KHV is solely based on the results of the PCR examinations.

Occurrence of KHVD in Japan and Practical Diagnosis of the Disease

The mortality among common carp cultured in net pens at Lake Kasumigaura increased since the beginning of October 2003, when the water temperature of the lake was 16-18℃. The fish swam lethargically near the water surface. There were no marked external signs in the most of the affected fish, but a whitish mucous-like substance on the body surface, redness of the fin and body, fin rot, and discoloration of the gill with some necrosis were sometimes observed.

The mortality was over 60% in the most severe cases, especially in larger carp over 2 years old. The losses of the cultured carp were estimated to be 660 tons by early November and reached approximately 1,200 tons by mid-November, which is approximately 1/4 of the annual production of the lake.

External parasites, such as *Chilodonella*, *Trichodina*, and *Gyrodactylus*, were sometimes seen on the necrotic gill of the affected fish. Marked histopathological changes were observed in the gill of the diseased carp (Fig. 2).

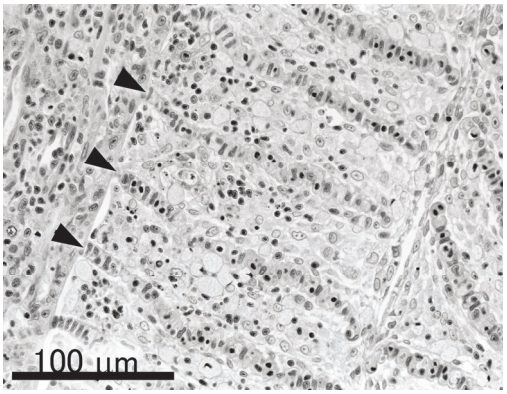

Fig. 2. A tissue section of the gill from affected common carp cultured at Lake Kasumigaura. Arrowheads indicate secondary lamellae, H&E stain.

The secondary lamellae were often fused with hyperplastic branchial epithelium where cell necrosis or the infiltration of lymphocytes were often observed. Congestion and hemorrhage were sometimes observed. In some cases, the branchial tissues were severely degraded and numerous bacteria were seen in those lesions. These histopathological changes are similar to those of the previous reports (Hedrick *et al*., 2000; Tu *et al*., 2004). Unlike previous report (Hedrick *et al*., 2000), nuclear changes characterized by hypertrophy and margination of chromatin were rarely observed. No bacteria was dominantly isolated from the kidney of affected fish were observed on trypticase soy agar.

The PCR test for KHV revealed specific bands amplified by the methods of Gray *et al*. (2002) and Gilad *et al*. (2002) (Fig. 3).

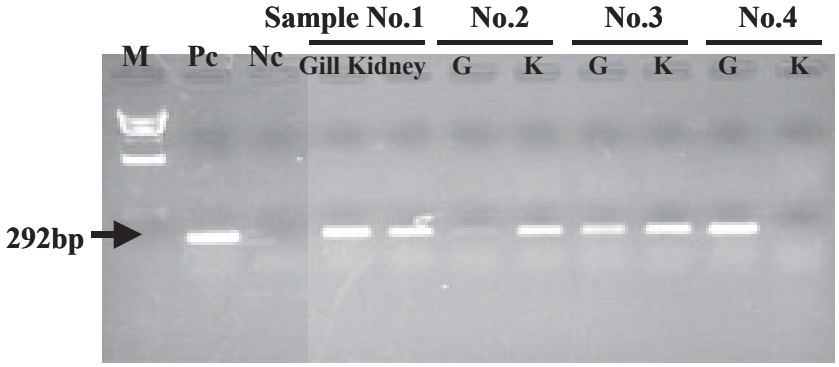

Fig. 3. Gel electrophoresis of the products (292bp) amplified with the primer set sph I-5 of Gray *et al.* (2002) from the extracted sample of gill and kidney of affected fish cultured in Lake Kasumigaura. M: marker, Pc: positive control, Nc: negative control. 1% agarose gel stained with ethidium bromide.

The sequence of the amplicon by the to the sequence deposited in the GenBank at accession no. AY568951, and that by the primer set of Gilad *et al*. showed 99% matching to AF411803.

The Ministry of Agriculture, Forestry and Fisheries (MAFF) of Japan officially announced the first case of KHV in Japan on November 2, 2003. At that time, the PFESs did not have sufficient equipment and technology for conducting PCR tests for

primer set of Gray *et al*. was identical
KHV detection. Therefore, at first, most of the PCR tests for KHV were carried out at the NRIA.

The NRIA gave a short training course of PCR for KHV for the research staff of PFESs in mid-November. PCR inspection for KHV was then established in Japan in accordance with the procedure in the guidelines.

Evidence of the Presence of KHV before the Outbreak at the Lake Kasumigaura

Independently of the Lake Kasumigaura outbreak, a massive loss in excess of 10,000 carp occurred in some rivers and a lake in Okayama Prefecture in late May to mid-July 2003. However, in November 2003, the NRIA detected KHV DNA by PCR in samples of diseased fish stored in a freezer. This demonstrates that KHV had been introduced into Japan by May 2003, well before the Lake Kasumigaura outbreak.

The Spread of KHV in the Fall of 2003

The KHV-infected common carp cultured in Lake Kasumigaura had been transferred to the other areas in Japan before the first detection of the KHV, and, therefore, the virus had spread as a result. In some facilities, mortalities of carp with KHV were reported. However, in many cases, KHV was detected in carp when there was no mortality in the facility. This could be attributed to the fact that the water temperature was gradually decreasing at the time. By the end of 2003, the NRIA has diagnosed 529 individuals of 87 cases, and KHV had been found in 23 out of 47 prefectures in Japan. However, half of the cases had no obvious relations with the Lake Kasumigaura.

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